

## Variation for Seedling Root Architecture in the Core Collection of Pea Germplasm

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### ABSTRACT

Root growth is an important component of plant growth but has received little attention by plant breeders because of difficulties associated with root observation. Improved root architecture and production are likely to improve pea (*Pisum sativum* L.) production because they are often grown on marginal land and suffer from increased disease pressure and poor fertility. The objective of this study was to quantify the phenotypic variation in seedling root production among *Pisum* germplasm and to classify the root architecture observed. Seed of 330 accessions from the core collection of *Pisum* germplasm were germinated and grown under artificial conditions for 14 d and their root characters analyzed. Root systems were scanned and digitized by the WinRHIZO program. Data collected included taproot length, shoot length, and root and shoot dry weight. Data generated by WinRHIZO included total root length, surface area, average root diameter, and root volume. Taproot length ranged from 181 to 433 mm, while root and shoot dry weight ranged from 6 to 57 mg and 13 to 104 mg, respectively. Total root length ranged from 54 to 399 cm, root surface area ranged from 9 to 75 cm<sup>2</sup>, and root volume ranged from 0.11 to 1.12 cm<sup>3</sup>. Seed weight was significantly correlated with all root characters with the exception of taproot length. Shoot and root dry weight were positively correlated with total root length, average root diameter, and root volume. PI 261631, an accession from Spain, produced the greatest total root length, had a root:shoot weight ratio of 0.79, and the largest root volume (1.12 cm<sup>3</sup>). Plant breeders will be able to use the wide variation for root characters in their crossing and selection programs to modify root traits during cultivar development.

PLANT BREEDING has largely ignored root production because of difficulty in observing root growth under natural conditions. Relatively few and sporadic reports of variation for root characters and their genetic control are available despite their importance to plant growth. Roots are responsible for sessile plant growth and function to absorb nutrients and water from the soil. Roots of leguminous crops such as pea also function as a host to *Rhizobium leguminosarum* through symbiotic associations. The *Rhizobium* form nodules in root hairs and fix gaseous nitrogen (N<sub>2</sub>) into ammonia (NH<sub>3</sub>) a form the plant can use. The fertility benefit from N<sub>2</sub> fixation is an added benefit from the use of pea and other legumes in crop rotations throughout the world.

Pea is traditionally produced in marginal areas and receives little attention from producers compared with other crops such as wheat, *Triticum aestivum* L. (Muehl-

bauer, 1996). Many pea diseases common in production areas affect root growth and function, thereby limiting productivity (Kraft and Pflieger, 2001, p. 67). Tolerance to many root diseases has been attributed to rapid root growth and production of large root mass; however, improved root growth parameters have not always been correlated with increased productivity (Kraft and Boge, 2001). However, analysis of seedling root characters has been shown to relate well to root production under field conditions (Kraft and Boge, 2001; Veitenheimer and Gritton, 1984). Prolific root growth is not only expected to provide an advantage to the plant in the presence of pathogens, but also under conditions where herbicide damage may occur (Ali-Kahn and Snoad, 1977). Many herbicides applied to the pea crop have potential to damage plant growth when taken up by roots. Rapid growth and proliferation of lateral roots would provide plants an opportunity to avoid severe damage and possibly death.

Improvement in root architecture has been the result of indirect selection for disease tolerance or overall productivity. Studies of root growth and function have been conducted on pea over the last 75 yr; however, reports are few and intermittent in the literature (Jean, 1928; Kraft and Boge, 2001; Veitenheimer and Gritton, 1984; Zobel, 1974). Jean (1928) demonstrated that heritable variation exists in pea and that root growth was directly related to the phenotype of the shoot. Veitenheimer and Gritton (1984) studied 42 freezing and canning pea genotypes and found significant variability in the root and shoot characters evaluated; however, this variability was not related to previously expressed tolerance to root rot caused by *Aphanomyces euteiches* Drechs. f. sp. *pisi* W.F. Pfender & D.J. Hagedorn.

Root characters are controlled by approximately one-third of the plant genome (Zobel, 1975) and should not be ignored by plant breeders. An understanding of the relationship between various root traits and plant productivity is necessary before improvements can be achieved. A comprehensive summary of the root traits which vary among pea germplasm and the degree of this variation is lacking in the literature. The objectives of this study were (i) to quantify measurable root traits among a diverse collection of *Pisum* accessions and (ii) to classify the architectural variation in pea seedling root systems.

### MATERIALS AND METHODS

#### Seed Source and Growth Conditions

Seed of 330 pea accessions from the core collection of *Pisum* germplasm was harvested from field plots in 1997 and stored at 4°C and low humidity before analysis (McPhee and Muehlbauer, 2001). The accessions were chosen at random from a

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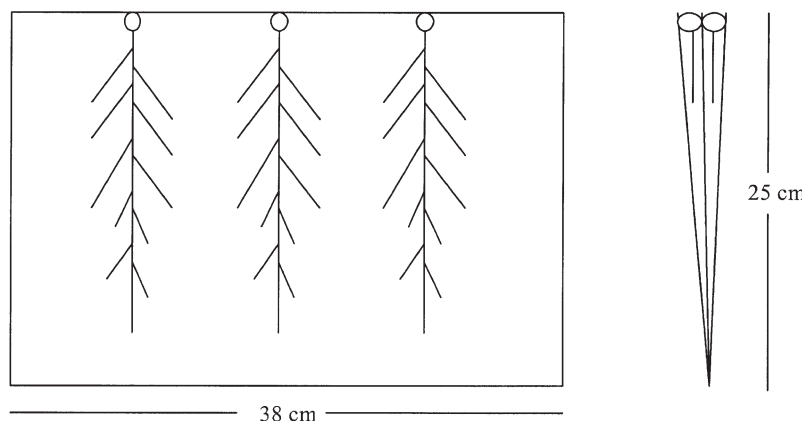


Fig. 1. Diagrammatic representation of pea root production in layered germination paper.

total of 504 accessions comprising the core collection. Ten seeds of uniform and average size were selected from each accession and scarified with a razor blade to allow uniform imbibition. The seeds were placed on moist germination paper (25 × 19 cm) (Hoffman Manufacturing, Inc., Albany, OR) with the radicle oriented downward. The germination paper was then folded in half covering the seeds completely and rolled inside a sheet of wax paper to maintain moisture content. The seeds were then placed in a germination chamber at 20°C with high humidity for 72 h.

After germination, six uniform and representative seedlings were removed and evenly placed near the upper edge of a full sheet (25 × 38 cm) of germination paper (three seedlings per each of two sheets stacked on top of each other) in landscape orientation (Fig. 1). A third sheet was laid on top covering the upper three seedlings. All three sheets were secured together using large paper clips. A narrow sheet of wax paper was folded across the bottom of the germination paper to contain any roots exceeding 25 cm in length. The seedlings were returned to the germination chamber and allowed to grow for 11 d in the presence of light. Acrylic sheets were cut to 25 × 40 cm and used to hold the germination paper bundles upright and rigid. At the end of the 2-wk period the seedlings were removed and three uniform and representative seedlings selected for analysis. Distilled water without nutrients was added as needed to maintain saturation in the germination paper. The presence and intensity of lighting was not considered important for root growth at the seedling stage and was not quantified in this study.

### Scanning and Analysis

Digital imagery identical to that used by Kraft and Boge (2001) was used to study root characters of pea. This methodology is easy to use and allows reliable prediction of phenotypic response under field conditions. Roots and shoots were excised from the seed piece at the cotyledonary node and their lengths recorded. The root mass was spread on the glass surface of a Hewlett-Packard Scanjet 6100C (Hewlett-Packard, Boise, ID) fitted with a transparency adaptor and connected to a Pentium computer with an AMD-K6 3D processor (Advanced Micro Devices, Inc., Sunnyvale, CA), 40 gigabyte hard drive, 128 MB RAM, and Windows 98. All lateral roots were spread to minimize overlapping roots. A two dimensional image of the root was collected using the WinRHIZO software package (v. 5.0, Regent Instruments, Inc., Quebec, QC, Canada). Once the image was collected, the root and shoot were placed in individual coin envelopes and dried at 50°C for 48 h and their dry weights recorded.

Scanned images were analyzed by the WinRHIZO program

for total root length, surface area, root volume, and average diameter. These variables are calculated on the basis of a common set of parameters and are autocorrelated. Before scanning the three root systems selected for analysis, they were photographed with a Sony Mavica, MVC-FD71 digital camera (Sony Corporation, Tokyo, Japan). The digital photographs were used to characterize root morphology and assign visual ratings to the roots. Data analysis was conducted by the SAS statistical package (v. 8.01, SAS Institute, Inc., Cary, NC). Coefficients of variation for the root characters analyzed ranged from 6.5 to 19.8 indicating uniformity among the seedlings chosen for analysis.

## RESULTS AND DISCUSSION

### Variation in Root Biomass

Taproot length among the 330 pea accessions ranged from 181 to 433 mm with a mean of 335 mm (Table 1). Root and shoot dry weight ranged continuously from 6.5 to 56.6 mg and 12.5 to 104.5 mg, respectively. Similarly, values for all root characters derived from analysis with WinRHIZO also varied continuously. Interaction between various environmental stimuli and the genetic potential of each individual accession is expected to have contributed to the continuous distribution. Conclusions regarding inheritance of root characters would be speculative at best given the broad range of variability represented by the accessions tested. Root dry weight ranged from 6 to 57 mg and was highly correlated with total root length and other values generated from WinRHIZO (Table 2). Root and shoot dry weight were highly and significantly correlated ( $r = 0.68$ ,  $p < 0.0001$ ). On the basis of regression analysis, root dry weight for accessions producing the least shoot dry weight varied little from

Table 1. Minimum, maximum and mean values for 10 root and shoot characters from the core collection of *Pisum* germplasm.

Character	Minimum	Maximum	Mean
Shoot length (mm)	59	380	246 ± 2.52
Taproot length (mm)	181	433	335 ± 1.48
Shoot dry weight (mg)	13	104	55 ± 0.60
Root dry weight (mg)	6	57	24 ± 0.27
Total root biomass (mg)	20	146	79 ± 0.36
Root:shoot ratio	0.20	0.89	0.45 ± 0.002
Total root length (cm)	54	399	174 ± 2.04
Root surface area (cm <sup>2</sup> )	9	75	30 ± 0.36
Average diameter (mm)	0.39	0.81	0.56 ± 0.002
Total root volume (cm <sup>3</sup> )	0.11	1.12	0.42 ± 0.006

Table 2. Correlation among several root characters of 330 *Pisum* germplasm accessions.

Related root characters	Total biomass	Seed yield	Shoot dry weight	Root dry weight	Total root length
Root dry weight	0.29***	0.35***	0.65***		
Total root length	0.21***	0.26***	0.46***	0.74***	
Average root diameter	0.16***	0.20***	0.33***	0.34***	−0.13***
Total root volume	0.24***	0.31***	0.58***	0.86***	0.77***

\*\*\* Statistically significant at  $p < 0.001$ .

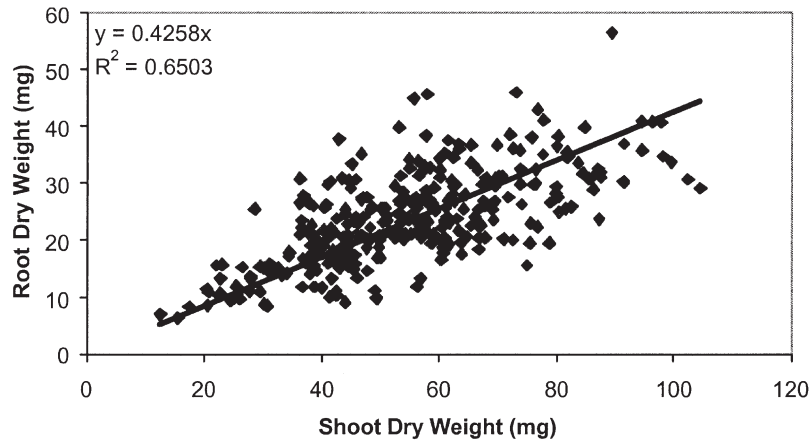


Fig. 2. Scatter plot of root dry weight against shoot dry weight for 330 PI accessions from the core collection of *Pisum* germplasm.

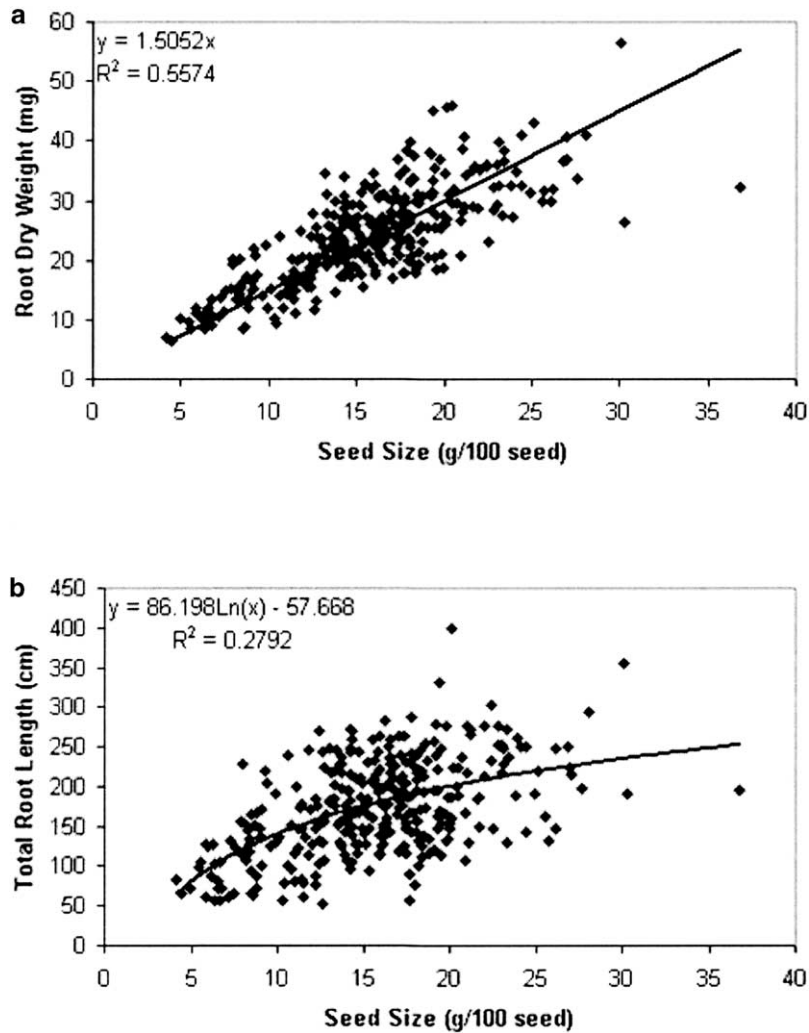


Fig. 3. Scatter plot of root dry weight (a) and total root length (b) plotted against average seed size for 330 PI accessions from the core collection of *Pisum* germplasm.

expected values while accessions producing greater total biomass deviated much more widely from the expected (Fig. 2).

Ratio of root dry weight to shoot dry weight ranged from 0.20 to 0.89 (Table 1) and agrees with values reported by Jensen (1985) and Ali-Khan and Snoad (1977). Shoot length was positively correlated with taproot length ( $r = 0.27$ ,  $p < 0.0001$ ). However, root dry weight, total root volume, average root diameter, and root surface area were negatively correlated with shoot dry weight. Total root length was not correlated with shoot length ( $r = 0.01$ ,  $p = 0.78$ ). Since total root length is primarily determined by the number and length of lateral roots, shoot length was not expected to be a reliable predictor of total root length or mass. Jean (1928) reported that root penetration was directly related to height of the above ground portion; however, this is related to overall taproot length and not branch number and length. Accessions with the greatest root:shoot dry weight ratio were PI 103058, PI 137119, PI 180693, PI 197044, and PI 261631.

Seed size is known to affect seedling vigor and is important for early radicle growth. Seed size (determined in field-grown seed) among 330 pea accessions ranged from 4.2 to 36.8 g 100 seed<sup>-1</sup>. Fig. 3a and b show a positive trend for root biomass production and total root length with increasing seed size. Specific accessions such as PI 261631 and PI 197044 produced the greatest total root length among the accessions studied and are characterized by relatively large seed. The opposite relationship (small seed size and small root production) was apparent in PI 174921, PI 175231, and PI 280609 (Fig. 3). Several accessions (PI 166084, PI 249645, PI 181800, PI 180695, PI 137119, and PI 180693) with seed size less than 15.0 g 100 seed<sup>-1</sup> produced total root length in excess of one standard deviation above the mean, 228.2 cm. Further study and hybridization with these accessions may yield small-seeded genotypes producing larger root mass.

Correlation analysis showed that seed weight was positively correlated with shoot dry weight, root dry weight, total root length and root volume (data not shown). These results corroborate previous studies showing that the cotyledon is important in seedling establishment (Ali-Kahn and Snoad, 1977). Correlations between total biomass and seed production determined in a separate study (McPhee and Muehlbauer, 2001) showed positive relationships with root dry weight, total root length, average root diameter and total root length (Table 2). These data suggest that increased productivity may be related to root production; however, detailed experiments using a subset of accessions should be conducted to verify this relationship.

Although it is difficult to assign a specific improvement status (landrace, breeding line, finished variety, etc.) to each accession, it is quite evident from accompanying passport data that accessions with the largest root mass have benefited from genetic improvement. Origin of accessions in the top and bottom 10% total root length

**Table 3. Distribution by country of origin for pea germplasm accessions ranking among the top and bottom 10% for total root production.**

Top 10%		Bottom 10%	
Country	Number of accessions	Country	Number of accessions
Australia	PI 314797	Afghanistan	PI 116944
Canada	PI 137119		PI 125839
Ethiopia	PI 195631		PI 125840
	PI 196877		PI 134271
	PI 226564		PI 198735
	PI 358628		PI 207508
France	PI 263027		PI 220174
	PI 263031		PI 222071
Germany	PI 180692		PI 222117
	PI 180693		PI 223526
	PI 180702	Austria	PI 125673
Guatemala	PI 195404	China	PI 141966
	PI 200755		PI 210558
Honduras	PI 197044	Czech Republic	PI 393489
Hungary	PI 320972	Ethiopia	PI 156647
India	PI 240515		PI 358613
	PI 347461	Former Soviet Union	PI 280609
Malaysia	PI 378160		PI 429849
Mexico	PI 319374	Germany	PI 288263
Netherlands	PI 244093	Hungary	PI 324700
	PI 244121		PI 324702
	PI 244191		PI 324703
	PI 261671		PI 413685
New Zealand	PI 371796	India	PI 179970
Poland	PI 285715	Iran	PI 227457
	PI 285739	Mali	PI 271115
Spain	PI 261631	Nepal	PI 174921
Syria	PI 179451		PI 175231
Turkey	PI 174320	Netherlands	PI 261677
United Kingdom	PI 269802	Pakistan	PI 274307
USA	PI 206848	USA	PI 206861
	PI 236492	Yugoslavia	PI 184128
Yugoslavia	PI 357290		PI 251051

is presented in Table 3 and shows that accessions with small root mass originate from countries near the Middle East, the center of origin for pea. One-third of the accessions with the least root growth originated from Afghanistan and one-half of the accessions originated from countries near the Middle East. One-fourth of the accessions with the least root production originated from developed countries, while two-thirds of those with large root production originated from developed countries.

### Relation to Disease Resistance

Tolerance to soil-borne fungal pathogens such as *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F.R. Jones) W.C. Snyder & H.N. Hans. and *A. euteiches* is important in nearly all pea production regions. Previous studies have found little or no correlation between various root characters and resistance ratings (Veitenheimer and Gritton, 1984; Kraft and Boge, 2001). Kraft and Boge (2001) reported no significant difference in disease severity between large- and small-rooted genotypes. Those genotypes with large root systems did, however, have greater root area later in the growing season in the presence of disease than genotypes with small root systems.

Root character data from the current study showed little correlation with previously determined resistance scores to *F. solani* f. sp. *pisi* obtained from the Germplasm Resources Information Network, Beltsville, MD. Taproot length was negatively correlated with root rot scores,  $r = -0.12$ , while root dry weight and total root



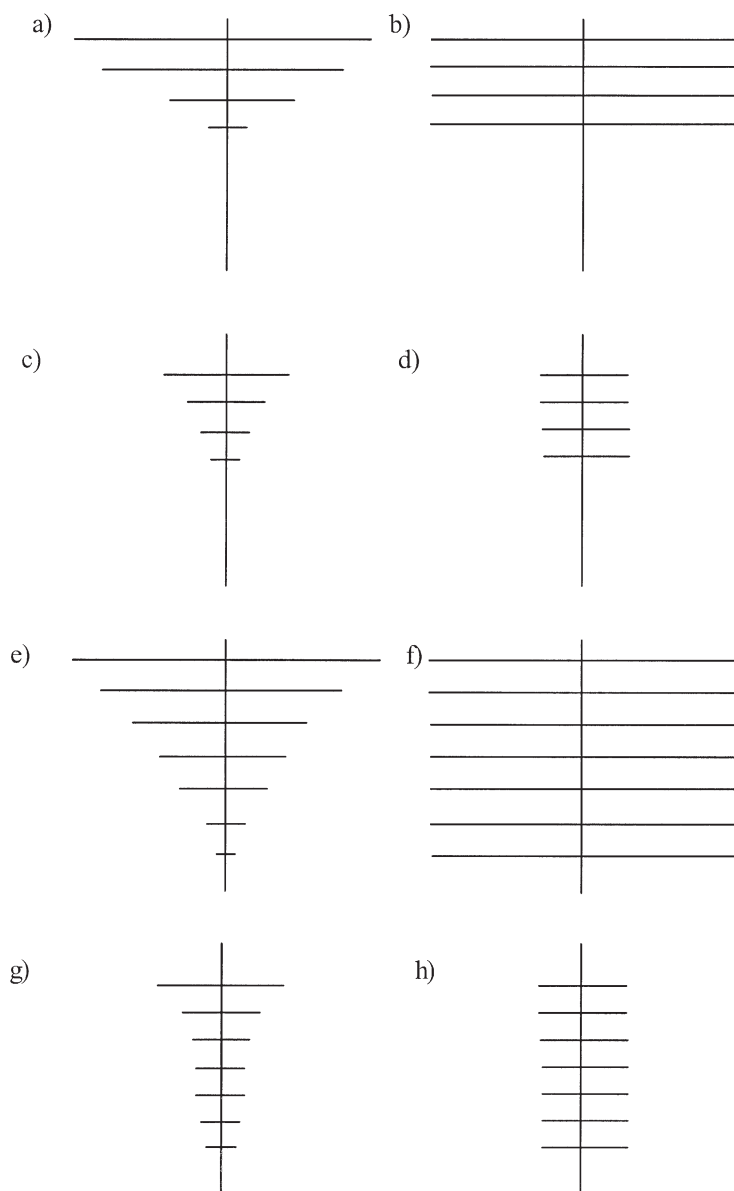


Fig. 4. Pictorial representation of a proposed description system of pea seedling root architecture including eight possible cross classifications of triangular (a, c, e, g) and rectangular (b, d, f, h) overall shape, proximal (a, b, c, d) and distal (e, f, g, h) distribution of laterals along the taproot and long (a, b, e, f) and short (c, d, g, h) lateral root length.

length were positively correlated with root rot scores,  $r = 0.37$  and  $0.19$ , respectively. The findings of Kraft and Boge (2001) and the current results indicate that additional factors beyond root morphology and biomass production are responsible for field tolerance to soil-borne diseases.

### Classification of Root Morphology

Pea root systems are characterized by a taproot with numerous lateral branches which can undergo further branch producing secondary and tertiary roots as well as root hairs. Seedling root systems (2 wk old) analyzed in this study were limited to the taproot and primary lateral branches. Although adventitious roots have been observed in pea, conditions of this study did not promote

their development. Root architecture among the accessions analyzed in the current study differed in the number, length, and distance between laterals (data not presented). The shape of the root system for many of the accessions was triangular, but several were more rectangular (i.e., first appearing laterals were relatively short). Root architecture of PI 116844 appeared heterogeneous among the three seedlings. The first had laterals distributed over the full length of the taproot, all with equal length (rectangular shape overall). The second and third seedlings had triangular shaped architecture, but differed in distribution of laterals along the taproot and the overall length of the laterals. This variation within an accession, which can be expected in landraces, has been observed in other species (Lynch 1995) and pro-

**Table 4. PI accessions exemplifying each of the eight (a–h) architectural classes of root morphology identified in pea.**

Morphology class	Shape	Distribution	Relative length	PI accessions
A	triangle	proximal	long	PI 269778 PI 365420 PI 413684
B	rectangle	proximal	long	PI 179970 PI 203944 PI 271038
C	triangle	proximal	short	PI 174921 PI 279823 PI 413685
D	rectangle	proximal	short	PI 134271 PI 280609 PI 324703
E	triangle	distal	long	PI 241593 PI 261631 PI 320972
F	rectangle	distal	long	PI 180695 PI 197044 PI 358640
G	triangle	distal	short	PI 240518 PI 261633 PI 280603
H	rectangle	distal	short	PI 125673 PI 251051 PI 261677

vides additional support for genetic control over root production and architecture.

A classification system for root morphology beyond that of basic anatomical description is needed in pea as a reference for future study. Basal roots have been reported in other legume species (Lynch 1995) but were not observed on the roots systems analyzed in this study. A system proposed here describes seedling root architecture on the basis of triangular and rectangular overall shape cross classified with (i) the distribution of lateral root along the taproot (proximal vs. distal) and (ii) lateral root length (long vs. short) (Fig. 4). These eight classes would serve to categorize all root systems in a general sense yet allow latitude for variation within each class. Specific accessions exemplifying each class are listed in Table 4. The dominant architecture observed in this study had a triangular shape, distal distribution of lateral roots and long lateral roots (Fig. 4e); however, length of the lateral roots varied widely. Root systems representing all types of architecture were observed at various frequency.

## CONCLUSIONS

Root growth and production is important in crop production. Water absorption and nutrient uptake are vital and can be limited in many production regions, especially marginal areas where legumes are traditionally grown. This is the first report summarizing seedling root characters and architecture among a large and diverse collection of pea germplasm. Results show wide variation for root biomass production and architecture in pea. Selection for increased root biomass in pea will likely require a recurrent selection approach. Plant breeders with the assistance of modern digital technology can now include improvement of root characters as an objective in their breeding programs. Adoption of the uniform classification system for seedling root architecture proposed here will strengthen broad understanding of data collected in a variety of conditions and laboratories. All root character data has been submitted to the Germplasm Resource Information Network (GRIN) and can be viewed at <http://www.ars-grin.gov/npgs/>; verified 3 May 2005.

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